Barrier dysfunction in the skin allergy

AUTHOR(S):

Egawa, Gyohei; Kabashima, Kenji

CITATION:

Egawa, Gyohei ...[et al]. Barrier dysfunction in the skin allergy. Allergology International 2018, 67(1): 3-11

ISSUE DATE:

2018-01

URL:

http://hdl.handle.net/2433/230801

Allergology International 67 (2018) 3–11

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Invited Review Article

Barrier dysfunction in the skin allergy

Gyohei Egawa [a, **], Kenji Kabashima [a, b, c, *]

[a] Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan
[b] Singapore Immunology Network (SIgN) and Institute of Medical Biology, Agency for Science, Technology and Research (A*STAR), Biopolis, Singapore
[c] PRESTO, Japan Science and Technology Agency, Saitama, Japan

ARTICLE INFO

Article history:
Received 22 September 2017
Received in revised form
30 September 2017
Accepted 4 October 2017
Available online 16 November 2017

Keywords:
Atopic dermatitis
Barrier function
Cornified envelope
Stratum corneum
Tight junction

Abbreviations:
AD, atopic dermatitis; CE, cornified envelope; FLG, filaggrin gene; KLK, kallikrein; NMF, natural moisturizing factor; PCA, pyrrolidine carboxylic acid; SC, stratum corneum; SG, stratum granulosum; SPR, small proline-rich protein; TG, transgultaminase; TJ, tight junction; UCA, urocanic acid

ABSTRACT

The skin is continuously exposed to external pathogens, and its barrier function is critical for skin homeostasis. Previous studies have shown that the barrier dysfunction is one of the most predisposing factors for the development of skin allergic diseases such as atopic dermatitis. In this article, we summarize how the physical barrier of the skin is organized and review its link to the pathomechanism of skin allergic diseases. We describe the formation of the SC barrier in terms of the following five categories: 1) filaggrin metabolism; 2) cornified envelope; 3) intercellular lipids; 4) corneodesmosome; and 5) corneocyte desquamation. New approaches to restoring the skin barrier function are also discussed.

Introduction

The skin covers the entire body and protects us from various kinds of external stimuli. An impaired epidermal barrier allows the enhanced penetration of external antigens and readily induces skin inflammation. This facilitates the interaction of external antigens with local immune cells and may lead to systemic immune responses.[1] This is called the “outside-to-inside” hypothesis, and it explains the association between skin barrier dysfunction and an increased risk of developing allergic diseases, including atopic dermatitis (AD), asthma, food allergies, and allergic rhinitis.[2,3] In addition, it is evident that persistent skin inflammation, in turn, causes further attenuation of the skin barrier, suggesting the existence of an exacerbation loop between the skin barrier and skin immunity (the “outside-to-inside-and-back-to-outside” hypothesis).[4,5] These observations suggest that maintaining the skin barrier function is important not only for effective management of allergic diseases but also for preventing their development.

The barrier function of the skin is largely dependent on the stratum corneum (SC), the outermost layer of the epidermis (Fig. 1A). The SC is formed through a course of tightly regulated processes of keratinocyte differentiation called keratinization.[6] Keratinization is achieved by keratinocytes passing through four cell layers of the epidermis: the stratum basale, the stratum spinosum, the stratum granulosum (SG), and the SC. In the SG, keratinocytes start to produce two membrane-circumscribed granules: keratohyalin granules and lamellar bodies (Fig. 1B). Keratohyalin granules contain intracellular components of the SC (such as filaggrin [FLG], loricrin, and keratin filaments), whereas lamellar bodies contain extracellular components (such as lipids, corneodesmosin, and kallikreins). In the SC, keratinocytes are flattened

* Corresponding author. Department of Dermatology, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawahara, Sakyo, Kyoto 606-8507, Japan.
** Corresponding author.
E-mail addresses: gyohei@kuhp.kyoto-u.ac.jp (G. Egawa), kaba@kuhp.kyoto-u.ac.jp (K. Kabashima).
Peer review under responsibility of Japanese Society of Allergology.

https://doi.org/10.1016/j.alit.2017.10.002

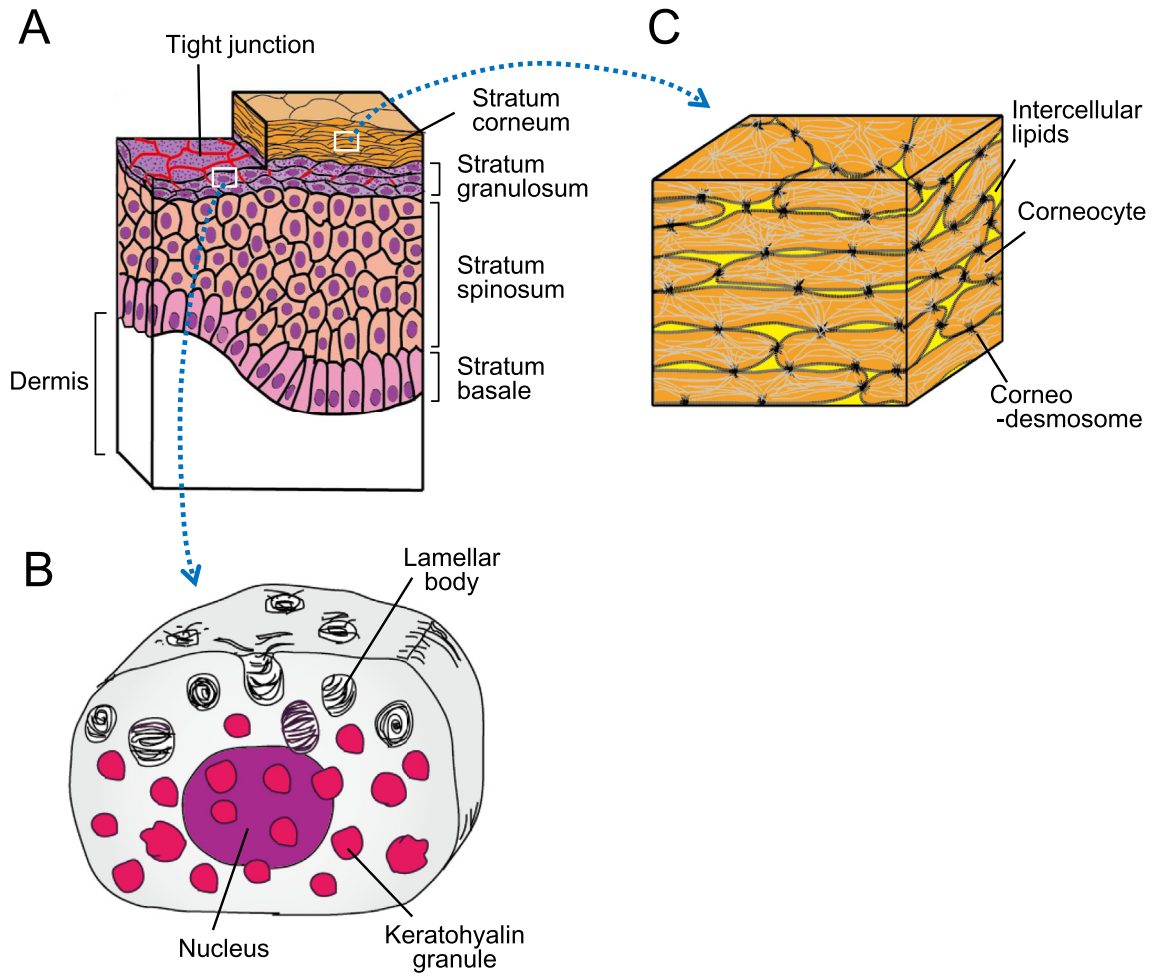

Fig 1. **A**, The structure of the epidermis. The red line represents tight junctions in the stratum granulosum. **B**, Magnified view of the cell in the stratum granulosum. **C**, The "bricks and mortars" structure of the stratum corneum.

and denucleated (which are then called as corneocytes) and simultaneously, corneocyte cell membranes are replaced by a specific barrier structure called a cornified envelope (CE). At the transition from the SG to the SC, lamellar bodies are secreted into the intercellular space of corneocytes and fill it up with lipids. These structures are often described as bricks (corneocytes) and mortar (intercellular lipids) (Fig. 1C).

Here, we describe the formation of the SC barrier in terms of the following five categories to review their link to the pathogenesis of skin allergic diseases: 1) FLG metabolism; 2) CE; 3) intercellular lipids; 4) corneodesmosome; and 5) corneocyte desquamation. The genes involved in each process are listed in Table 1. This review is an updated version of a similar article that has published in the Journal of Allergy and Clinical Immunology.[3]

Filaggrin metabolism

FLG and its metabolites are key components for maintaining normal skin barrier function (Fig. 2).[7,8] In the SG, FLG is produced as FLG polymer (profilaggrin), in which 10–12 repeats of FLG monomer are linked, and stored in keratohyalin granules. At the transition from the SG to the SC, profilaggrin is cleaved to generate FLG monomers by proteases such as CAP1/Prss8 and SASPase/ASPRV1.[9,10] FLG monomers bind to keratin filaments and this keratin-FLG bundle is a fundamental structure in corneocytes. At the upper layer of the SC, FLG is re-dissociated from keratin filaments to further metabolism. In this process, the citrullination of FLG and keratin1 by peptidylarginine deiminase is considered essential.[11] The released FLG monomers are degraded to free amino acids, including glutamine, arginine, and histidine, and then converted into urocanic acid (UCA) and pyrrolidine carboxylic acid (PCA). This process is mediated by other sets of proteases, including caspase14, calpain1, and bleomycin hydrolase.[12,13] UCA is an important ultraviolet-absorbing chromophore in the SC and also contributes to maintaining the acidic pH of the skin.[14] Recent study has shown that UCA significantly reduced costimulatory molecule expression on dendritic cells and increased their ability to induce a regulatory T cells.[15] In contrast, PCA is a major constituent of natural moisturizing factors (NMFs), which are responsible for retaining water in the SC. Therefore, FLG and its metabolites assume a manifold role in the barrier function of the SC. Gene targeting studies have revealed that FLG-deficient mice demonstrate a reduced SC barrier function with enhanced sensitization.[16] Furthermore, on a proallergic BALB/c background, FLG-deficient mice develop spontaneous dermatitis.[17]

Filaggrin deficiency in the skin allergy

AD is the most common inflammatory skin disease, and has multiple etiologies. Over the last decade, data from both experimental models and patients have highlighted the primary pathogenic role of skin barrier deficiency in AD.[18–20] Particularly, loss-of-

Table 1
A list of genes which regulate stratum corneum formation.

	Gene	Gene symbol	Function	Associated human disease	Knockout mice phenotype	Reference
FLG metabolism	Filaggrin	FLG	Keratin filaments aggregation	Ichthyosis vulgaris, **AD**	Skin barrier deficiency Spontaneous dermatitis	16–18,21
	Filaggrin2	FLG2	Similar to FLG?	AD?		29
	Cap1/Prss8	PRSS8	Cleave proFLG to FLG		Skin barrier deficiency	9
	SASpase	ASPRV1	Cleave proFLG to FLG	AD?	SC dehydration	10
	Peptidylarginine deiminase	PADI	Citrullilation of FLG			11
	Caspase14	CASP14	FLG metabolism		Skin barrier deficiency	12
	Calpain1	CAPN1	FLG metabolism			13
	Bleomycin hydrolase	BLMH	FLG metabolism		Penetrant ring-tail dermatitis	13
Formation of Cornified envelope	Involucrin	IVL	Scaffold of CE		No skin phenotype	36
	Envoplakin	EVPL	Plakin family		No skin phenotype	37
	Periplakin	PPL	Plakin family		No skin phenotype	38
	Loricrin	LOR	Reinforce CE		Shiny skin	40
	Small proline-rich protein	SPRR	Reinforce CE			96
	Transglutaminase 1	TGM1	Crosslink CE proteins	ARCI1	Skin barrier deficiency	41,97
	Transglutaminase 3	TGM3	Crosslink CE proteins		Skin barrier deficiency	98
	Transglutaminase 5	TGM5	Crosslink CE proteins	Peeling skin syndrome 2		42
Intercellular lipid- lamellae formation	12R-lipoxygenase	ALOX12B	Ceramide processing	ARCI2	Skin barrier deficiency Neonatal death	46,99
	Epidermal lipoxygenase 3	ALOX3E	Ceramide processing	ARCI3	Skin barrier deficiency Neonatal death	46,100
	ATP-binding cassettesubfamily A member 12	ABCA12	Transport of lamellar body	ARCI 4A/4B (Harlequin ichthyosis)	Skin barrier deficiency	47,101
	Tmem79/mattrin	TMEM79	Secretion of lamellar bodies	AD?	Spontaneous dermatitis	48,49
Corneodesmosome	Desmoglein1	DSG1	Cadherin family	**SAM syndrome**		51
	Desmocollin1	DCN1	Cadherin family		Skin barrier deficiency	102
	Plakoglobin	JUP	Armadillo family	Naxos disease	Embryonic lethal	103
	Plakophilin	PKP	Armadillo family	Skin fragility syndrome	PKP3-deficient mice develop dermatitis	104
	(Envoplakin)	EVPL	Plakin family		No skin phenotype	37
	(Periplakin)	PPL	Plakin family		No skin phenotype	38
	Corneodesmosin	CDSN	Support the corneodesmosome adhesion	**Peeling skin syndrome 1**	Skin barrier deficiency Neonatal death	105
Corneocyte desquamation	Kallikrein5	KLK5	Serine protease		Skin inflammation (when overexpressed)	106
	Kallikrein7	KLK7	Serine protease	AD?	Skin inflammation (when overexpressed)	107
	Kallikrein14	KLK14	Serine protease			108
	Lympho-epithelial Kazal-type-related inhibitor (LEKTI)	SPINK5	Serine protease inhibitor	**Netherton syndrome,** AD?	Neonatal death due to dehydration	61–63,109
	Protease-activated receptor 2	PAR2	Receptor on keratinocytes		Altered skin immune response	110

The diseases that represent allergic manifestations are shown in bold.

function mutations in the *FLG* gene are strongly associated with the development of AD as well as with ichthyosis vulgaris.[18,21] The prevalence of *FLG* mutations in AD patients ranges from 25 to 50% in the Northern European and Asian populations.[22,23] In addition, genome-wide association studies (GWAS) among individuals with European, African, Japanese, and Latino ancestry have identified 31 risk loci of AD, to date, and among them, the mutation in *FLG* is proved to be the strongest risk factor.[24] These observations indicate the major contribution of FLG-deficiency in AD pathogenesis.

Although mutations in *FLG* are common in Northern European and Asian subjects, *FLG* mutations are less common in Southern Europe,[25] and are even absent in some African countries.[26,27] A recent study showed that the expression of another skin barrier protein, FLG2, is reduced in the epidermis of AD patients.[28] Further, a nonsense mutation in the *FLG2* gene was shown to be associated with persistent AD patients of African ancestry.[29] The biological function of FLG2 remains to be elucidated, but its structure, pattern of expression, and biological properties are very similar to FLG. Therefore, FLG2 may also play important roles in skin barrier integrity. We must also note the possibility that FLG deficiency might be compensable under a tropical climate.[30]

Formation of the cornified envelope

The cornified envelope (CE) is a specific barrier structure formed beneath the cell membrane of corneocytes (Fig. 3).[31] The CE consists of highly crosslinked insoluble proteins and the extracellular lipids anchoring on it. This structure acts as a vital physical barrier to the SC.

The assembly of the CE starts in the upper layer of the stratum spinosum. In response to the elevation of intracellular Ca^{2+} levels, keratinocytes produce envoplakin, periplakin, and involucrin. Envoplakin and periplakin form heterodimers, and, together with involucrin, they accumulate beneath the plasma membrane.[32] These three proteins are crosslinked to each other by transglutaminase (TG) 1 and TG5.[33] Involucrin acts as a scaffold of the CE,

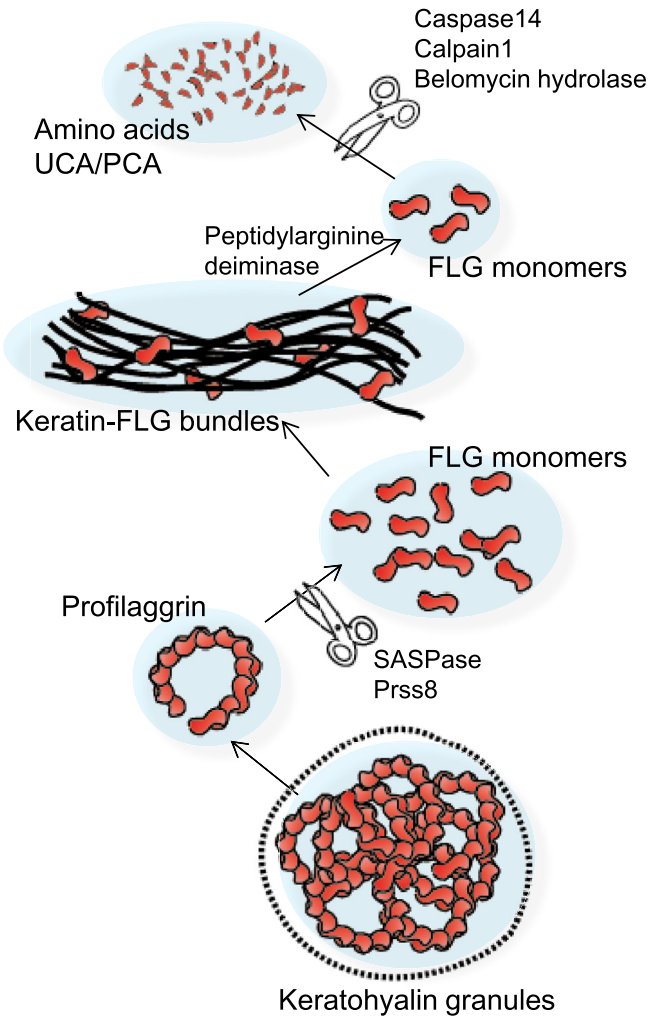

Fig 2. Schema of the FLG metabolic process. In the stratum granulosum, profilaggrins are stored in keratohyalin granules and then cleaved into FLG monomers. FLG monomers bind to keratin filaments in corneocytes. At the upper layer of the SC, FLG monomers are released from keratins and cleaved into free amino acids, followed by conversion into PCA and UCA.

while plakin dimers act as a binding site of keratin filaments and combine them with desmosomal proteins. Importantly, since plakin proteins are tightly crosslinked to the involucrin scaffold, desmosomes and keratin filaments are rigidly linked on the CE, which confers mechanical stability to corneocytes.

In the SG, loricrin and small proline-rich (SPR) protein families are produced. These proteins are crosslinked by TG3 and translocate to the cell periphery; next they crosslink onto the pre-existing scaffold of involucrin by TG1 and TG5.[34] To reinforce the CE, this crosslinking is repeated and as the result, up to 80% of the CE protein consists of loricrin. TG1 also combines extracellular ceramide lipids onto the scaffold of involucrin, and eventually, the ceramides replace the lipid bilayer of the plasma membrane.[35] This step is further described in section "Formation of intercellular lipid lamellae".

Cornified envelope formation in the skin allergy

Despite the ubiquitous presence of involucrin, envoplakin, and periplakin in the CE, single knockout mice of these genes do not show any obvious skin abnormalities.[36–38] The triple knockout of these three genes results in abnormal CE formation with reduced lipid content and decreased mechanical integrity, but the skin barrier function is normal (possibly compensated by reduced desquamation of corneocytes).[39] Similarly, loricrin-deficient mice exhibit only a subtle phenotype, with shiny skin at birth and reduced CE stability.[40] These studies suggest that CE proteins are redundant and indicate the existence of strong compensatory mechanisms. In accordance with this notion, no mutations of the genes of CE components have been linked to the pathogenesis of skin allergic diseases thus far.

The CE is abnormal or even absent with TG1-deficiency, in which severe ichthyosiform erythroderma (autosomal recessive congenital ichthyosis [ARCI] 1) develops.[41] In addition, TG5 deficiency causes peeling skin syndrome 2, which represents as superficial acral skin peeling occurring at the junction between the SG and the SC.[42] These facts suggest the non-redundant role of TGs in the formation of CE; however, the association between genetic mutation in TGs and skin allergic diseases has not been reported.

Formation of intercellular lipid lamellae

The intercellular lipids (the "mortar") are also an integral component of the SC barrier (Fig. 3). They consist of a heterogeneous mixture of ceramides, free fatty acids, and cholesterol in a roughly 1:1:1 M ratio. These lipids are produced in the SG and stored in lamellar bodies, and are subsequently secreted into extracellular space in the transition to the SC. In the ceramide fraction alone, over 300 distinct species have been identified from human SC.[43] Among them, omega-hydroxyceramide is indispensable because it is conjugated to the involucrin scaffold by TG1 and covers the surface of corneocytes. Using this ceramide as a template, periodic sheets of lipid lamellae are formed in the intercellular space of corneocytes.[44]

Intercellular lipid lamellae formation in the skin allergy

Several defects in ceramide-processing enzymes have been linked to the etiology of barrier-deficient skin diseases. 12R-lipoxygenase (encoded in the *ALOX12B* gene) and epidermal lipoxygenase-3 (encoded in the *ALOXE3* gene) are both essential for the generation of omega-hydroxyceramide.[45] Defect in these proteins causes congenital ichthyosis (ARCI2, and ARCI3, respectively).[46] The skin manifestations of ARCI2 and ARCI3 are less severe than those of ARCI1, probably because the protein layer of the CE is formed in these diseases.

The transmembranal transport of lamellar bodies is conducted by a lipid transporter called ATP-binding cassette subfamily A member 12 (ABCA12).[47] Mutations of this gene result in moderate (ARCI4A) to severe (ARCI4B, also known as harlequin ichthyosis) congenital ichthyosis, suggesting an essential role of lamellar body contents in normal cornification. Recently, transmembrane protein 79/mattrin (Tmem79/Matt) was identified to be involved in the secretion of lamellar body contents.[48,49] Tmem79 is a five-transmembrane protein that is localized to lamellar bodies, and Tmem79-deficient mice exhibit spontaneous dermatitis with elevated serum IgE, which resembles human AD manifestations. In addition, a meta-analysis of AD patients revealed that a missense mutation of the *TMEM79* gene has a small but significant association with AD.[49] These findings suggest that the abnormality of the lamellar body function and subsequent intercellular lipid layer dysformation might result in barrier deficiency in some AD patients.

Structure of corneodesmosome

The cell adhesion of corneocytes is dependent on the desmosome apparatus, called the corneodesmosome (Fig. 3). The

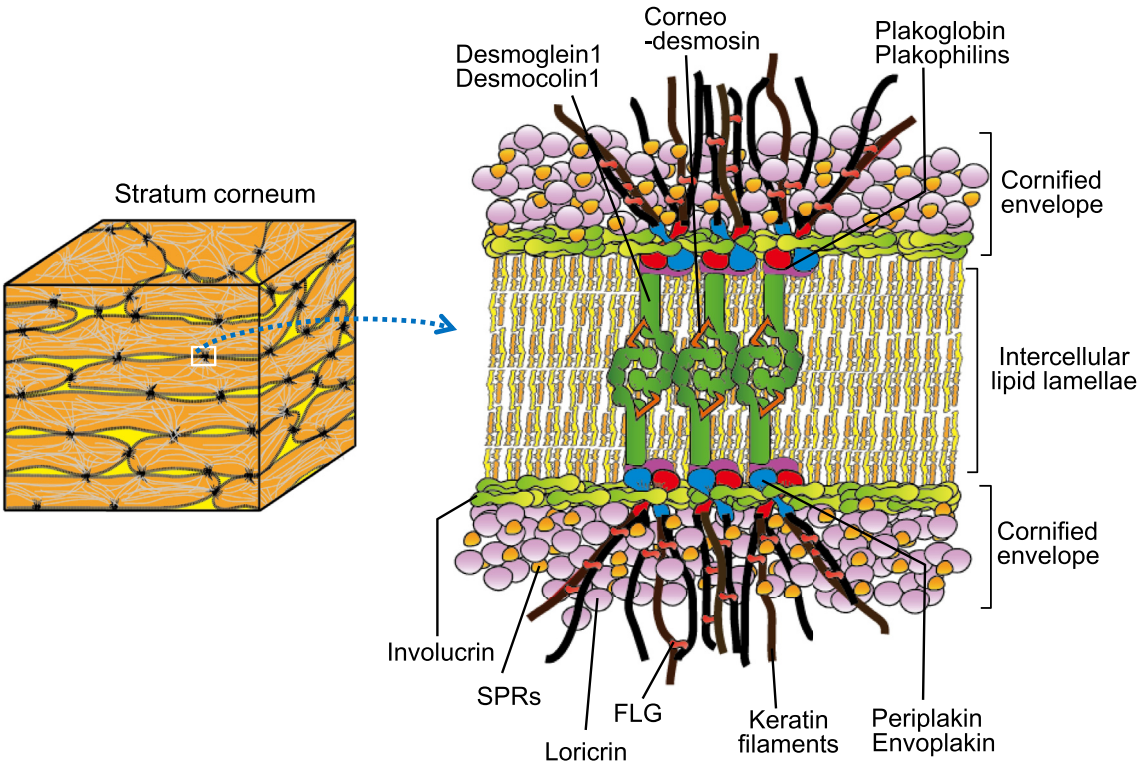

Fig 3. The structures of the cornified envelope and corneodesmosome. Involucrin forms the scaffold and is reinforced by loricrin and SRRs. Envoplakin-periplakin heterodimers conjugate keratin filaments.

desmosome is composed of three protein families: desmosomal cadherin, armadillo proteins, and plakins. In the corneodesmosome, desmoglein 1 and desmocollin 1 (cadherin family) interact with plakoglobin and plakophilins (armadillo proteins), which attach to envoplakin and periplakin. As described above, envoplakin and periplakin heterodimers are crosslinked to the involucrin scaffold, and bind keratin filaments on its C-terminus. The corneodesmosin is another important modulator of corneodesmosomal adhesion.[50] It is stored in the lamellar bodies and secreted into the intracellular space of the SC, and interacts with cadherin proteins to support their adhesion.

Corneodesmosome formation in the skin allergy

Abnormality of the corneodesmosome is prone to cause hyperdesquamation of corneocytes, which may lead to skin barrier defect and subsequent skin inflammation. A recent study revealed that the homozygous mutation of desmoglein 1 results in severe dermatitis (erythroderma), accompanied by palmoplantar keratoderma, hypotrichosis, and increased serum IgE (EPKHE, also known as severe dermatitis, multiple allergies, and metabolic wasting [SAM] syndrome).[51] Importantly, EPKHE patients often have multiple food allergies. In contrast, the homozygous mutation of the corneodesmosin causes peeling skin syndrome 1, characterized by dermatitis, severe pruritus, food allergies, repeated episodes of angioedema and urticaria, asthma, and increased serum IgE.[52]

Corneocyte desquamation

At the surface layer of the SC, corneocytes are constantly shed. This phenomenon is called desquamation, and it is an important process to maintain the SC homeostasis. Corneocyte desquamation is mainly regulated by a proteolytic cascade of kallikrein (KLK)-related peptidases, such as KLK5, KLK7, and KLK14.[53] The activity of these proteases is pH-dependent and is enhanced when the pH in the SC is elevated. Their activity is also strictly regulated by a cocktail of protease inhibitors, including lymphoepithelial Kazal-type 5 serine protease inhibitor (LEKTI) encoded by the serine protease inhibitor Kazal-type 5 (*SPINK5*).[54] KLKs and LEKTI are stored in lamellar bodies and secreted into the intercellular space at the SG-SC interface.

Corneocyte desquamation in the skin allergy

In AD patients, the skin surface pH is increased, at least partially due to the decreased production of UCA derived from FLG (Fig. 2).[55] As such, KLK activity is often enhanced in the AD skin. This condition is thought to induce an adverse effect on the SC barrier through a multimodal mechanism (Fig. 4). First, KLKs cleave corneodesmosomal cadherins and promote corneocyte desquamation. Second, KLKs activate protease-activated receptor (PAR)-2, a G-protein-coupled receptor on keratinocytes. Upon activation, PAR-2 signals lead to suppression of lamellar body secretion via the downregulation of lipid processing enzymes.[56] Finally, activated KLKs increase the generation of interleukin (IL)-1α and IL-1β, whose preforms are abundantly stored in the cytosol of corneocytes. Indeed, IL-1 cytokines are increased in the SC of AD patients and their enhanced production is associated with FLG deficiency.[57]

Two genetic polymorphisms that result in increased KLKs activity have been linked to AD pathogenesis: gain-of-function mutations in *KLK7* and loss-of-function mutations in *SPINK5*. The 4bp insertion polymorphism of *KLK7* was first reported in the UK,[58] but their association with AD was not found in a French AD cohort study.[59] *SPINK5* is known as the gene responsible for Netherton syndrome in which patients display a broad range of allergic manifestations, such as AD-like dermatitis, food allergies, asthma, hay fever, and

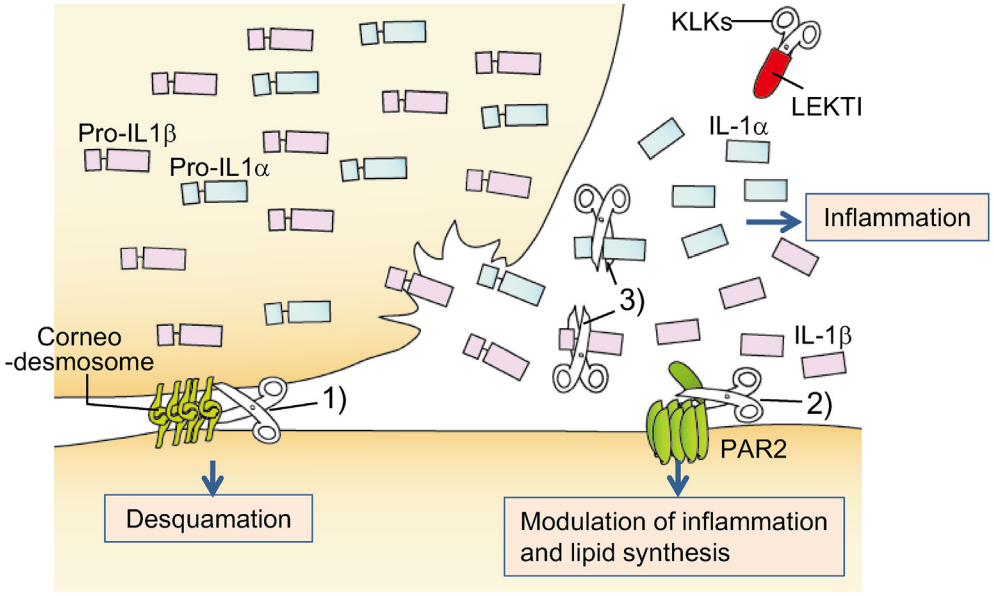

Fig. 4. Kallikrein function in the SC. KLKs cleave; 1) corneodesmosomal cadherins and promote desquamation; 2) PAR2 to activation that regulates lipid synthesis and immune responses; and 3) IL-1 preforms. IL-1 preforms are stored in the cytosol of corneocytes and escape to intercellular space upon cellular damage.

markedly elevated serum IgE levels.[60] A significant association of polymorphism in *SPINK5* with AD was reported in the UK and Asian populations,[61–63] but not found in the French population.[59]

Tight junction in the skin allergy

In addition to the SC, tight junctions (TJs) are structures that are essential to the integrity of the skin barrier. In the skin, TJs seal adjacent keratinocytes in the SG (Fig. 1A) and act as a barrier for water and solutes.[64] TJs are composed of transmembrane proteins, such as the claudin and occludin family, and several cytosolic scaffold proteins, including zonulae occludens (ZOs). The indispensable role of TJs in skin homeostasis was first demonstrated by claudin1-deficient mice that died within 24 h of birth with severe dehydration.[65] Importantly, these mice had no abnormalities in the production of SC components. A recent study with AD model mice showed that the expression of TJ proteins was suppressed with skin inflammation, but was not affected by FLG deficiency.[66]

In humans, the claudin1 expression is reduced in the non-lesional skin of AD patients, and the association of claudin1 polymorphism with AD susceptibility has been reported.[67] These observations suggest that an impairment in TJs contributes to the barrier dysfunction observed in AD patients. Since most of the skin is covered with the SC, TJs seem to act as the second line of defense against external pathogens; however, TJs must be the primary barrier structure in skin appendages, such as hair follicles and sweat glands, because the SC is absent in these areas. Indeed, it is well known that hair follicles are important shunt routes into the skin for drugs and chemicals.[68] In accordance with this notion, widespread eruptive infections with *herpes simplex virus* or *molluscum contagiosum virus*, which enter the body through hair follicles, sometimes occur as a complication of AD.[69,70] These facts suggest that the skin appendages are the “security holes” of the skin, particularly in AD patients with TJ deficiency.

Immunological modulation of skin barriers

Accumulating evidence suggests that immune cells influence skin integrity through the production of cytokines.[71,72] Although complex interaction of immune cells creates AD skin lesions, the immunopathogenesis of AD is characterized by Th2-skewed responses.[73,74] Previous studies have shown that IL-4 and IL-13, the two major Th2 cytokines, downregulate the production of 1) FLG and keratins, 2) the CE components (loricrin and involucrin), 3) cell adhesion molecules (desmogleins, ZO-1), and 4) ceramide lipids. IL-31, another Th2 cytokine dominantly produced by Th2 cells, also downregulates FLG expression.[75] Furthermore, a recent study has shown that IL-33, an alarmin that is abundantly stored in keratinocytes, has the potency to downregulate FLG expression as well.[76] The original purpose of these immunological modulations against skin integrity may be to facilitate the desquamation and replacement of damaged corneocytes; however, to achieve this, dysregulation of the skin barrier is essential. A series of these modulations may cause problems, particularly in AD patients. The exacerbation loop between congenital barrier deficiency and immunogenic barrier deficiency leads to the formation of chronic, persistent skin inflammation in AD.

Barrier dysfunction is leading pathogenesis of skin allergy

It is now evident that epicutaneous antigens are strong sensitizer of allergic disorders. Mouse studies have demonstrated that food allergy and asthma can be induced via epicutaneous sensitization and are enhanced under disrupted skin barrier. In human, sequential acquisition of allergic diseases (atopic march) are frequently observed in both AD and some genodermatoses, such as Netherton syndrome (mutation in *SPINK5*), peeling skin syndrome 1 (*Corneodesmosin*) and SAM syndrome (*Desmoglein1*) (Table 1, bold), which strongly suggests that skin barrier deficiency contributes to the development of atopic march. Eosinophilic esophagitis is another chronic immune disorder that is associated with hypersensitivity to food, and has recently been linked to the mutations in *Calpain 14* (*CAPN14*), a protease specifically expressed in the esophagus.[77] An in-vitro expriment showed that overactivation of CAPN14 results in loss of Desmoglein1.[78] These studies demonstrate that barrier deficiency in mucosal epithelium also contribute to the induction of allergic disorders. Recent clinical trials have shown that epicutaneous antigen exposure induces

sensitization while oral antigen consumption induces immune tolerance.[79,80]

In the presence of barrier defects in the SC, foreign antigens readily penetrate into the epidermis and activate innate immune receptors and pattern recognition receptors. This results in the production of Th2-promoting cytokines, such as IL-33, IL-25 and thymic stromal lymphoproteins (TSLP), which are produced by skin resident cells. Animal studies have demonstrated an essential role for TSLP in the epicutaneous induction of food allergy with AD-like skin lesions. Increased TSLP in the epidermis elicits the accumulation of basophils into the skin that promote Th2-cytokine responses.[81] In addition, TSLP signaling on epidermal Langerhans cells may be important for IgE production during the epicutaneous sensitization to food allergens.[82] The suppressive effect of TSLP on FLG expression was also confirmed by the human skin engrafted on immunocompromised mice.[83]

It is noteworthy that cytokine profiles in patients with ichthyosis vulgaris, who are carrying FLG mutation, have an IL-17-dominant and low expression level of Th2-related cytokines.[84] This immune profile resemble to psoriasis patients, rather than AD. These findings suggest that barrier dysfunction alone might not induce Th2 immune profiles.

Conclusion – therapeutic approach to restore skin barrier function

Skin barrier deficiency and excessive immune responses are two sides of the same coin in skin allergic diseases, and each is highly relevant to the other.[85] Therefore, therapeutics targeting the skin barrier function, as well as immunosuppressive drugs, can be considered important in the effective management of skin allergy. Recently, two groups investigated whether protecting the skin barrier with a moisturizer during the neonatal period prevents the development of AD.[86,87] They reported that moisturizer treatment at an early stage of life resulted in 32–50% less AD prevalence. These results suggest that to avoid percutaneous sensitization in the neonatal period by reinforcing the skin barrier function is a promising strategy to prevent AD.

The potency of FLG replacement therapies has been demonstrated. This approach includes the application of 1) read-through drugs, 2) drugs that enhance FLG production, 3) the FLG monomer itself, and 4) FLG metabolites. Read-through drugs are able to skip the nonsense mutation of the *FLG* gene, which may be applicable to both heterozygous and homozygous *FLG* mutation carriers. Candidate drugs are antimicrobial peptides, such as gentamicin and PTC124 (Ataluren). These drugs are currently tried in clinical trials for other genetic diseases.[88,89] In contrast, the drugs that enhance FLG production are only considered applicable to patients with heterozygous FLG mutations. Candidates include agonists of peroxisome proliferator-activated receptors (PPARs),[90] a serine-rich diet,[91] apigenin,[92] JTC801,[93] JTE-052,[83] and urea.[94] All of these drugs have been demonstrated to induce keratinocyte differentiation and increase FLG levels. Further studies are necessary, however, because their efficacy has only been assessed *in vitro* or in animal models.

Intensive research to identify promising candidates to enhance the skin barrier function is ongoing[95] and is expected to lead to better management of skin allergic diseases, including AD, in the near future.

Acknowledgement

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Conflict of interest

The authors have no conflict of interest to declare.

References

1. Brunner PM, Silverberg JI, Guttman-Yassky E, Paller AS, Kabashima K, Amagai M, et al. Increasing comorbidities suggest that atopic dermatitis is a systemic disorder. *J Invest Dermatol* 2017;**137**:18–25.
2. Spergel JM, Paller AS. Atopic dermatitis and the atopic march. *J Allergy Clin Immunol* 2003;**112**:S118–27.
3. Egawa G, Kabashima K. Multifactorial skin barrier deficiency and atopic dermatitis: essential topics to prevent the atopic march. *J Allergy Clin Immunol* 2016;**138**:350–8. e1.
4. Elias PM, Steinhoff M. "Outside-to-inside" (and now back to "outside") pathogenic mechanisms in atopic dermatitis. *J Invest Dermatol* 2008;**128**:1067–70.
5. Otsuka A, Nomura T, Rerknimitr P, Seidel JA, Honda T, Kabashima K. The interplay between genetic and environmental factors in the pathogenesis of atopic dermatitis. *Immunol Rev* 2017;**278**:246–62.
6. Matsui T, Amagai M. Dissecting the formation, structure and barrier function of the stratum corneum. *Int Immunol* 2015;**27**:269–80.
7. O'Regan GM, Sandilands A, McLean WI, Irvine AD. Filaggrin in atopic dermatitis. *J Allergy Clin Immunol* 2009;**124**:R2–6.
8. Levin J, Friedlander SF, Del Rosso JQ. Atopic dermatitis and the stratum corneum part 1: the role of filaggrin in the stratum corneum barrier and atopic skin. *J Clin Aesthet Dermatol* 2013;**6**:16–22.
9. Leyvraz C, Charles R-P, Rubera I, Guitard M, Rotman S, Breiden B, et al. The epidermal barrier function is dependent on the serine protease CAP1/Prss8. *J Cell Biol* 2005;**170**:487–96.
10. Matsui T, Miyamoto K, Kubo A, Kawasaki H, Ebihara T, Hata K, et al. SASPase regulates stratum corneum hydration through profilaggrin-to-filaggrin processing. *EMBO Mol Med* 2011;**3**:320–33.
11. Nachat R, Méchin M-C, Takahara H, Chavanas S, Charveron M, Serre G, et al. Peptidylarginine deiminase isoforms 1–3 are expressed in the epidermis and involved in the deimination of K1 and filaggrin. *J Invest Dermatol* 2005;**124**:384–93.
12. Hoste E, Kemperman P, Devos M, Denecker G, Kezic S, Yau N, et al. Caspase-14 is required for filaggrin degradation to natural moisturizing factors in the skin. *J Invest Dermatol* 2011;**131**:2233–41.
13. Kamata Y, Taniguchi A, Yamamoto M, Nomura J, Ishihara K, Takahara H, et al. Neutral cysteine protease bleomycin hydrolase is essential for the breakdown of deiminated filaggrin into amino acids. *J Biol Chem* 2009;**284**:12829–36.
14. Gibbs NK, Tye J, Norval M. Recent advances in urocanic acid photochemistry, photobiology and photoimmunology. *Photochem Photobiol Sci* 2008;**7**:655–67.
15. Leitch CS, Natafji E, Yu C, Abdul-Ghaffar S, Madarasingha N, Venables ZC, et al. Filaggrin-null mutations are associated with increased maturation markers on Langerhans cells. *J Allergy Clin Immunol* 2016;**138**:482–90. e7.
16. Kawasaki H, Nagao K, Kubo A, Hata T, Shimizu A, Mizuno H, et al. Altered stratum corneum barrier and enhanced percutaneous immune responses in filaggrin-null mice. *J Allergy Clin Immunol* 2012;**129**:1538–46. e6.
17. Saunders SP, Moran T, Floudas A, Wurlod F, Kaszlikowska A, Salimi M, et al. Spontaneous atopic dermatitis is mediated by innate immunity, with the secondary lung inflammation of the atopic march requiring adaptive immunity. *J Allergy Clin Immunol* 2016;**137**:482–91.
18. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;**38**:441–6.
19. Elias PM, Schmuth M. Abnormal skin barrier in the etiopathogenesis of atopic dermatitis. *Curr Allergy Asthma Rep* 2009;**9**:265–72.
20. Cork MJ, Danby SG, Vasilopoulos Y, Hadgraft J, Lane ME, Moustafa M, et al. Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol* 2009;**129**:1892–908.
21. Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 2006;**38**:337–42.
22. Sandilands A, Terron-Kwiatkowski A, Hull PR, O'Regan GM, Clayton TH, Watson RM, et al. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet* 2007;**39**:650–4.
23. Nomura T, Sandilands A, Akiyama M, Liao H, Evans AT, Sakai K, et al. Unique mutations in the filaggrin gene in Japanese patients with ichthyosis vulgaris and atopic dermatitis. *J Allergy Clin Immunol* 2007;**119**:434–40.
24. EArly G, Lifecourse EEEC, Australian AGCA, Consortium AAG. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nat Genet* 2015;**47**:1449.
25. Cascella R, Cuzzola VF, Lepre T, Galli E, Moschese V, Chini L, et al. Full sequencing of the FLG gene in Italian patients with atopic eczema: evidence of new mutations, but lack of an association. *J Invest Dermatol* 2011;**131**:982–4.
26. Winge M, Bilcha K, Lieden A, Shibeshi D, Sandilands A, Wahlgren CF, et al. Novel filaggrin mutation but no other loss-of-function variants found in Ethiopian patients with atopic dermatitis. *Br J Dermatol* 2011;**165**:1074–80.

27. Thawer-Esmail F, Jakasa I, Todd G, Wen Y, Brown SJ, Kroboth K, et al. South African amaXhosa patients with atopic dermatitis have decreased levels of filaggrin breakdown products but no loss-of-function mutations in filaggrin. *J Allergy Clin Immunol* 2014;**133**:280–2. e2.
28. Pellerin L, Henry J, Hsu C-Y, Balica S, Jean-Decoster C, Méchin M-C, et al. Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin. *J Allergy Clin Immunol* 2013;**131**:1094–102.
29. Margolis DJ, Gupta J, Apter AJ, Ganguly T, Hoffstad O, Papadopoulos M, et al. Filaggrin-2 variation is associated with more persistent atopic dermatitis in African American subjects. *J Allergy Clin Immunol* 2014;**133**:784–9.
30. Sasaki T, Furusyo N, Shiohama A, Takeuchi S, Nakahara T, Uchi H, et al. Filaggrin loss-of-function mutations are not a predisposing factor for atopic dermatitis in an Ishigaki Island under subtropical climate. *J Dermatol Sci* 2014;**76**:10–5.
31. Kalinin A, Marekov LN, Steinert PM. Assembly of the epidermal cornified cell envelope. *J Cell Sci* 2001;**114**:3069–70.
32. DiColandrea T, Karashima T, Määttä A, Watt FM. Subcellular distribution of envoplakin and periplakin insights into their role as precursors of the epidermal cornified envelope. *J Cell Biol* 2000;**151**:573–86.
33. Eckert RL, Sturniolo MT, Broome A-M, Ruse M, Rorke EA. Transglutaminase function in epidermis. *J Invest Dermatol* 2005;**124**:481–92.
34. Candi E, Tarcsa E, Idler WW, Kartasova T, Marekov LN, Steinert PM. Transglutaminase cross-linking properties of the small proline-rich 1 family of cornified cell envelope proteins integration with loricrin. *J Biol Chem* 1999;**274**:7226–37.
35. Nemes Z, Marekov LN, Fésüs L, Steinert PM. A novel function for transglutaminase 1: attachment of long-chain ω-hydroxyceramides to involucrin by ester bond formation. *Proc Natl Acad Sci U S A* 1999;**96**:8402–7.
36. Djian P, Easley K, Green H. Targeted ablation of the murine involucrin gene. *J Cell Biol* 2000;**151**:381–8.
37. Määttä A, DiColandrea T, Groot K, Watt FM. Gene targeting of envoplakin, a cytoskeletal linker protein and precursor of the epidermal cornified envelope. *Mol Cell Biol* 2001;**21**:7047–53.
38. Aho S, Li K, Ryoo Y, McGee C, Ishida-Yamamoto A, Uitto J, et al. Periplakin gene targeting reveals a constituent of the cornified cell envelope dispensable for normal mouse development. *Mol Cell Biol* 2004;**24**:6410–8.
39. Sevilla LM, Nachat R, Groot KR, Klement JF, Uitto J, Djian P, et al. Mice deficient in involucrin, envoplakin, and periplakin have a defective epidermal barrier. *J Cell Biol* 2007;**179**:1599–612.
40. Koch PJ, De Viragh PA, Scharer E, Bundman D, Longley MA, Bickenbach J, et al. Lessons from loricrin-deficient mice compensatory mechanisms maintaining skin barrier function in the absence of a major cornified envelope protein. *J Cell Biol* 2000;**151**:389–400.
41. Laiho E, Ignatius J, Mikkola H, Yee VC, Teller DC, Niemi K-M, et al. Transglutaminase 1 mutations in autosomal recessive congenital ichthyosis: private and recurrent mutations in an isolated population. *Am J Hum Genet* 1997;**61**:529–38.
42. Cassidy AJ, van Steensel MA, Steijlen PM, van Geel M, van der Velden J, Morley SM, et al. A homozygous missense mutation in TGM5 abolishes epidermal transglutaminase 5 activity and causes acral peeling skin syndrome. *Am J Hum Genet* 2005;**77**:909–17.
43. Masukawa Y, Narita H, Shimizu E, Kondo N, Sugai Y, Oba T, et al. Characterization of overall ceramide species in human stratum corneum. *J Lipid Res* 2008;**49**:1466–76.
44. Iwai I, Han H, den Hollander L, Svensson S, Öfverstedt L-G, Anwar J, et al. The human skin barrier is organized as stacked bilayers of fully extended ceramides with cholesterol molecules associated with the ceramide sphingoid moiety. *J Invest Dermatol* 2012;**132**:2215–25.
45. Krieg P, Fürstenberger G. The role of lipoxygenases in epidermis. *Biochim Biophys Acta* 2014;**1841**:390–400.
46. Eckl K-M, de Juanes S, Kurtenbach J, Nätebus M, Lugassy J, Oji V, et al. Molecular analysis of 250 patients with autosomal recessive congenital ichthyosis: evidence for mutation hotspots in ALOXE3 and allelic heterogeneity in ALOX12B. *J Invest Dermatol* 2009;**129**:1421–8.
47. Kelsell PD, Norgett EE, Unsworth H, Teh M-T, Cullup T, Mein CA, et al. Mutations in ABCA12 underlie the severe congenital skin disease harlequin ichthyosis. *Am J Hum Genet* 2005;**76**:794–803.
48. Sasaki T, Shiohama A, Kubo A, Kawasaki H, Ishida-Yamamoto A, Yamada T, et al. A homozygous nonsense mutation in the gene for Tmem79, a component for the lamellar granule secretory system, produces spontaneous eczema in an experimental model of atopic dermatitis. *J Allergy Clin Immunol* 2013;**132**:1111–20. e4.
49. Saunders SP, Goh CS, Brown SJ, Palmer CN, Porter RM, Cole C, et al. Tmem79/Matt is the matted mouse gene and is a predisposing gene for atopic dermatitis in human subjects. *J Allergy Clin Immunol* 2013;**132**:1121–9.
50. Lundström A, Serre G, Haftek M, Egelrud T. Evidence for a role of corneodesmosin, a protein which may serve to modify desmosomes during cornification, in stratum corneum cell cohesion and desquamation. *Arch Dermatol Res* 1994;**286**:369–75.
51. Samuelov L, Sarig O, Harmon RM, Rapaport D, Ishida-Yamamoto A, Isakov O, et al. Desmoglein 1 deficiency results in severe dermatitis, multiple allergies and metabolic wasting. *Nat Genet* 2013;**45**:1244–8.
52. Oji V, Eckl K-M, Aufenvenne K, Nätebus M, Tarinski T, Ackermann K, et al. Loss of corneodesmosin leads to severe skin barrier defect, pruritus, and atopy: unraveling the peeling skin disease. *Am J Hum Genet* 2010;**87**:274–81.
53. Brattsand M, Stefansson K, Lundh C, Haasum Y, Egelrud T. A proteolytic cascade of kallikreins in the stratum corneum. *J Invest Dermatol* 2005;**124**:198–203.
54. Deraison C, Bonnart C, Lopez F, Besson C, Robinson R, Jayakumar A, et al. LEKTI fragments specifically inhibit KLK5, KLK7, and KLK14 and control desquamation through a pH-dependent interaction. *Mol Biol Cell* 2007;**18**:3607–19.
55. Rippke F, Schreiner V, Doering T, Maibach HI. Stratum corneum pH in atopic dermatitis. *Am J Clin Dermatol* 2004;**5**:217–23.
56. Hachem J-P, Man M-Q, Crumrine D, Uchida Y, Brown BE, Rogiers V, et al. Sustained serine proteases activity by prolonged increase in pH leads to degradation of lipid processing enzymes and profound alterations of barrier function and stratum corneum integrity. *J Invest Dermatol* 2005;**125**:510–20.
57. Kezic S, O'Regan GM, Lutter R, Jakasa I, Koster ES, Saunders S, et al. Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency. *J Allergy Clin Immunol* 2012;**129**:1031–9. e1.
58. Vasilopoulos Y, Sharaf N, di Giovine F, Simon M, Cork MJ, Duff GW, et al. The 3′-UTR AACCins5874 in the stratum corneum chymotryptic enzyme gene (SCCE/KLK7), associated with atopic dermatitis; causes an increased mRNA expression without altering its stability. *J Dermatol Sci* 2011;**61**:131–3.
59. Hubiche T, Ged C, Benard A, Léauté-Labrèze C, McElreavey K, de Verneuil H, et al. Analysis of SPINK 5, KLK 7 and FLG genotypes in a French atopic dermatitis cohort. *Acta Derm Venereol* 2007;**87**:499–505.
60. Chavanas S, Bodemer C, Rochat A, Hamel-Teillac D, Ali M, Irvine AD, et al. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat Genet* 2000;**25**:141–2.
61. Walley AJ, Chavanas S, Moffatt MF, Esnouf RM, Ubhi B, Lawrence R, et al. Gene polymorphism in Netherton and common atopic disease. *Nat Genet* 2001;**29**:175–8.
62. Kato A, Fukai K, Oiso N, Hosomi N, Murakami T, Ishii M. Association of SPINK5 gene polymorphisms with atopic dermatitis in the Japanese population. *Br J Dermatol* 2003;**148**:665–9.
63. Zhao L, Di Z, Zhang L, Wang L, Ma L, Lv Y, et al. Association of SPINK5 gene polymorphisms with atopic dermatitis in Northeast China. *J Eur Acad Dermatol Venereol* 2012;**26**:572–7.
64. Kirschner N, Houdek P, Fromm M, Moll I, Brandner JM. Tight junctions form a barrier in human epidermis. *Eur J Cell Biol* 2010;**89**:839–42.
65. Furuse M, Hata M, Furuse K, Yoshida Y, Haratake A, Sugitani Y, et al. Claudin-based tight junctions are crucial for the mammalian epidermal barrier a lesson from claudin-1–deficient mice. *J Cell Biol* 2002;**156**:1099–111.
66. Yokouchi M, Kubo A, Kawasaki H, Yoshida K, Ishii K, Furuse M, et al. Epidermal tight junction barrier function is altered by skin inflammation, but not by filaggrin-deficient stratum corneum. *J Dermatol Sci* 2015;**77**:28–36.
67. De Benedetto A, Rafaels NM, McGirt LY, Ivanov AI, Georas SN, Cheadle C, et al. Tight junction defects in patients with atopic dermatitis. *J Allergy Clin Immunol* 2011;**127**:773–86. e7.
68. Otberg N, Patzelt A, Rasulev U, Hagemeister T, Linscheid M, Sinkgraven R, et al. The role of hair follicles in the percutaneous absorption of caffeine. *Br J Clin Pharmacol* 2008;**65**:488–92.
69. Blattner RJ. Molluscum contagiosum: eruptive infection in atopic dermatitis. *J Pediatr* 1967;**70**:997–9.
70. Lynch FW, Evans C, Bolin VS, Steves RJ. Kaposi's varicelliform eruption: extensive herpes simplex as a complication of eczema. *Arch Dermatol* 1945;**51**:129.
71. Howell MD, Kim BE, Gao P, Grant AV, Boguniewicz M, DeBenedetto A, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol* 2007;**120**:150–5.
72. Levin J, Fallon Friedlander S, Del Rosso JQ. Atopic dermatitis and the stratum corneum: part 3: the immune system in atopic dermatitis. *J Clin Aesthet Dermatol* 2013;**6**:37–44.
73. Honda T, Nomura T, Kabashima K. Advances in atopic dermatitis and urticarial in 2016. *J Allergy Clin Immunol* 2017;**140**:369–76.
74. Nomura T, Kabashima K, Miyachi Y. The panoply of αβT cells in the skin. *J Dermatol Sci* 2014;**76**:3–9.
75. Cornelissen C, Marquardt Y, Czaja K, Wenzel J, Frank J, Lüscher-Firzlaff J, et al. IL-31 regulates differentiation and filaggrin expression in human organotypic skin models. *J Allergy Clin Immunol* 2012;**129**:426–33. e8.
76. Seltmann J, Roesner LM, von Hesler F-W, Wittmann M, Werfel T. IL-33 impacts on the skin barrier by downregulating the expression of filaggrin. *J Allergy Clin Immunol* 2015;**135**:1659.
77. Kottyan LC, Davis BP, Sherrill JD, Liu K, Rochman M, Kaufman K, et al. Genome-wide association analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic disease. *Nat Genet* 2014;**46**:895–900.
78. Davis BP, Stucke EM, Khorki ME, Litosh VA, Rymer JK, Rochman M, et al. Eosinophilic esophagitis–linked calpain 14 is an IL-13–induced protease that mediates esophageal epithelial barrier impairment. *JCI insight* 2016;**1**.
79. Du Toit G, Sayre PH, Roberts G, Sever ML, Lawson K, Bahnson HT, et al. Effect of avoidance on peanut allergy after early peanut consumption. *N Engl J Med* 2016;**374**:1435–43.
80. Perkin MR, Logan K, Tseng A, Raji B, Ayis S, Peacock J, et al. Randomized trial of introduction of allergenic foods in breast-fed infants. *N Engl J Med* 2016;**374**:1733–43.

81. Noti M, Kim BS, Siracusa MC, Rak GD, Kubo M, Moghaddam AE, et al. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin–basophil axis. *J Allergy Clin Immunol* 2014;**133**:1390–9. e6.
82. Nakajima S, Igyártó BZ, Honda T, Egawa G, Otsuka A, Hara-Chikuma M, et al. Langerhans cells are critical in epicutaneous sensitization with protein antigen via thymic stromal lymphopoietin receptor signaling. *J Allergy Clin Immunol* 2012;**129**:1048–55. e6.
83. Amano W, Nakajima S, Kunugi H, Numata Y, Kitoh A, Egawa G, et al. The Janus kinase inhibitor JTE-052 improves skin barrier function through suppressing signal transducer and activator of transcription 3 signaling. *J Allergy Clin Immunol* 2015;**136**:667–77. e7.
84. Paller AS, Renert-Yuval Y, Suprun M, Esaki H, Oliva M, Huynh TN, et al. An IL-17–dominant immune profile is shared across the major orphan forms of ichthyosis. *J Allergy Clin Immunol* 2017;**139**:152–65.
85. Kabashima K. New concept of the pathogenesis of atopic dermatitis: interplay among the barrier, allergy, and pruritus as a trinity. *J Dermatol Sci* 2013;**70**: 3–11.
86. Horimukai K, Morita K, Narita M, Kondo M, Kitazawa H, Nozaki M, et al. Application of moisturizer to neonates prevents development of atopic dermatitis. *J Allergy Clin Immunol* 2014;**134**:824–30. e6.
87. Simpson EL, Chalmers JR, Hanifin JM, Thomas KS, Cork MJ, McLean WI, et al. Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. *J Allergy Clin Immunol* 2014;**134**:818–23.
88. Malik V, Rodino-Klapac LR, Viollet L, Wall C, King W, Al-Dahhak R, et al. Gentamicin-induced readthrough of stop codons in Duchenne muscular dystrophy. *Ann Neurol* 2010;**67**:771–80.
89. Hoffman EP, Bronson A, Levin AA, Si Takeda, Yokota T, Baudy AR, et al. Restoring dystrophin expression in duchenne muscular dystrophy muscle: progress in exon skipping and stop codon read through. *Am J Pathol* 2011;**179**: 12–22.
90. Zhang C, Gurevich I, Aneskievich BJ. Organotypic modeling of human keratinocyte response to peroxisome proliferators. *Cells Tissues Organs* 2012;**196**: 431–41.
91. Kim H, Lim Y-j, Park J-H, Cho Y. Dietary silk protein, sericin, improves epidermal hydration with increased levels of filaggrins and free amino acids in NC/Nga mice. *Br J Nutr* 2012;**108**:1726–35.
92. Hou M, Sun R, Hupe M, Kim PL, Park K, Crumrine D, et al. Topical apigenin improves epidermal permeability barrier homoeostasis in normal murine skin by divergent mechanisms. *Exp Dermatol* 2013;**22**:210–5.
93. Otsuka A, Doi H, Egawa G, Maekawa A, Fujita T, Nakamizo S, et al. Possible new therapeutic strategy to regulate atopic dermatitis through upregulating filaggrin expression. *J Allergy Clin Immunol* 2014;**133**:139–46. e10.
94. Grether-Beck S, Felsner I, Brenden H, Kohne Z, Majora M, Marini A, et al. Urea uptake enhances barrier function and antimicrobial defense in humans by regulating epidermal gene expression. *J Invest Dermatol* 2012;**132**: 1561–72.
95. Paller AS, Kabashima K, Bieber T. Therapeutic pipeline for atopic dermatitis: end of the drought? *J Allergy Clin Immunol* 2017;**140**:633–43.
96. Carregaro F, Stefanini ACB, Henrique T, Tajara EH. Study of small proline-rich proteins (SPRRs) in health and disease: a review of the literature. *Arch Dermatol Res* 2013;**305**:857–66.
97. Matsuki M, Yamashita F, Ishida-Yamamoto A, Yamada K, Kinoshita C, Fushiki S, et al. Defective stratum corneum and early neonatal death in mice lacking the gene for transglutaminase 1 (keratinocyte transglutaminase). *Proc Natl Acad Sci U S A* 1998;**95**:1044–9.
98. Bognar P, Nemeth I, Mayer B, Haluszka D, Wikonkal N, Ostorhazi E, et al. Reduced inflammatory threshold indicates skin barrier defect in transglutaminase 3 knockout mice. *J Invest Dermatol* 2014;**134**:105–11.
99. de Juanes S, Epp N, Latzko S, Neumann M, Fürstenberger G, Hausser I, et al. Development of an ichthyosiform phenotype in Alox12b-deficient mouse skin transplants. *J Invest Dermatol* 2009;**129**:1429–36.
100. Krieg P, Rosenberger S, de Juanes S, Latzko S, Hou J, Dick A, et al. Aloxe3 knockout mice reveal a function of epidermal lipoxygenase-3 as hepoxilin synthase and its pivotal role in barrier formation. *J Invest Dermatol* 2013;**133**: 172–80.
101. Yanagi T, Akiyama M, Nishihara H, Ishikawa J, Sakai K, Miyamura Y, et al. Self-improvement of keratinocyte differentiation defects during skin maturation in ABCA12-deficient harlequin ichthyosis model mice. *Am J Pathol* 2010;**177**: 106–18.
102. Chidgey M, Brakebusch C, Gustafsson E, Cruchley A, Hail C, Kirk S, et al. Mice lacking desmocollin 1 show epidermal fragility accompanied by barrier defects and abnormal differentiation. *Cell Biol* 2001;**155**:821–32.
103. Ruiz P, Birchmeier W. The plakoglobin knock-out mouse: a paradigm for the molecular analysis of cardiac cell junction formation. *Trends Cardiovasc Med* 1998;**8**:97–101.
104. Sklyarova T, Bonné S, D'hooge P, Denecker G, Goossens S, De Rycke R, et al. Plakophilin-3-deficient mice develop hair coat abnormalities and are prone to cutaneous inflammation. *J Invest Dermatol* 2008;**128**:1375–85.
105. Leclerc EA, Huchenq A, Mattiuzzo NR, Metzger D, Chambon P, Ghyselinck NB, et al. Corneodesmosin gene ablation induces lethal skin-barrier disruption and hair-follicle degeneration related to desmosome dysfunction. *J Cell Sci* 2009;**122**:2699–709.
106. Furio L, de Veer S, Jaillet M, Briot A, Robin A, Deraison C, et al. Transgenic kallikrein 5 mice reproduce major cutaneous and systemic hallmarks of Netherton syndrome. *J Exp Med* 2014;**211**:499–513.
107. Hansson L, Bäckman A, Ny A, Edlund M, Ekholm E, Hammarström BE, et al. Epidermal overexpression of stratum corneum chymotryptic enzyme in mice: a model for chronic itchy dermatitis. *J Invest Dermatol* 2002;**118**:444–9.
108. Stefansson K, Brattsand M, Ny A, Glas B, Egelrud T. Kallikrein-related peptidase 14 may be a major contributor to trypsin-like proteolytic activity in human stratum corneum. *Biol Chem* 2006;**387**:761–8.
109. Yang T, Liang D, Koch PJ, Hohl D, Kheradmand F, Overbeek PA. Epidermal detachment, desmosomal dissociation, and destabilization of corneodesmosin in Spink5-/-mice. *Genes Dev* 2004;**18**:2354–8.
110. Kawagoe J, Takizawa T, Matsumoto J, Tamiya M, Meek SE, Smith AJ, et al. Effect of protease-activated receptor-2 deficiency on allergic dermatitis in the mouse ear. *Jpn J Pharmacol* 2002;**88**:77–84.